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Tumor Viruses and Differentiation 1–14

Viruses, Protoviruses, Development, and Evolution

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Peyton Rous, a great scientist and exemplar, did not only discover Rous sarcoma virus (RSV)—or "chicken tumor I virus" as he called it—which caused spindle-celled sarcomas in sensitive chickens. He also found other chicken tumor viruses, among them a virus from chicken tumor VII which contained true cartilage and bone. This virus produced osteochondromas in sensitive birds. Rous stated [1]:

> Thus the agent, when brought into contact with the connective tissue in voluntary muscle, produces not an ordinary spindle-celled sarcoma, but a growth that elaborates cartilage and finally bone—that such an agent should bring about a differentiation ordinarily foreign to the tissue is very remarkable.

Thus the connection between tumor viruses and abnormal differentiation was noted at the very beginning of tumor virology.

In this paper I shall remind you of some history of the connection between RNA tumor viruses and differentiation and of RNA tumor viruses and movable genetic elements; summarize very briefly the relevant present knowledge of RNA tumor viruses; discuss the present status of the hypothesis of the existence of protoviruses in vertebrate genomes; and finally, discuss the possible role of movable genetic elements in differentiation and evolution.

I shall start with Barbara McClintock and maize. In the 1940s, McClintock started studying the mutable loci that arose after breakage-fusion-bridge cycles in maize. These cycles are occasioned by the presence of an inverted repeat in one of a pair of chromosomes followed during meiosis by crossing over and formation of a dicentric chromosome. In later mitoses, a break occurs in anaphase, followed by fusion of sister chromatids in later prophase. This fusion then results in a dicentric chromosome which again is broken and then fuses, etc. McClintock realized that after these cycles some factor was present that controlled the time or frequency of

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mutations and, thus, resulted in the appearance of sectored tissues. Her primary thesis stated [2]:

> Instability arises from alterations that do not directly alter the genes themselves, but affect the functioning of the genic components at or near the locus of alteration. The particular class to which a mutable locus belongs is related to the particular kind of chromatin substance that is present at or near the genic component in the chromosome. It is this material and the changes that occur to it that control the types and the rates of action of the genic components. Thus the basic mechanism responsible for a change at a mutable locus is considered to be one that is associated with a structural alteration of the chromatin materials at the locus. The mechanism that brings about these changes is related to the mitotic cycle; and it may involve alterations of both sister chromatids at the given locus. Some of these alterations may immediately result in the expression of an altered phenotype, a "gene mutation." Others produce modifications controlling the type of events that will occur at the locus in future cell and plant generations. Still others produce changes of a more extensive type, such as duplications and deficiencies of segments of chromatin in the vicinity of the locus.

She concluded that "this precise timing of somatic segregations effects a form of differentiation, for it brings about changes in the control of occurrence and time of occurrence of genic action." She called the elements causing the alterations "controlling elements."

Shapiro [3] has summarized the actions of the maize elements as creating somatic instability in chromosomes; inserting into coding or regulating sequences; adding new control sequences; and changing gene activity at later times. In light of these activities McClintock thought these controlling elements important in regulating cell differentiation.

The later discoveries of other cellular movable genetic elements and the resemblance of retroviruses in structure and behavior to cellular movable genetic elements again raises the question of the role of cellular movable genetic elements and retroviruses in normal cell differentiation. I am of the opinion that cellular movable genetic elements and retroviruses have little to do with the genetics of normal cell differentiation, but a lot to do with abnormal cell differentiation and evolution. Thus, I disagree with Barbara McClintock's hypothesis on the significance of these elements. However, I believe that tumor virus products and protooncogenes may have an important role in differentiation. In addition, I believe (and I understand McClintock did too) that cellular movable genetic elements have played an important role in evolution.

TUMOR VIROLOGY BEFORE 1965

After 1920 tumor virology left differentiation and approached virology as more tumor viruses were found and tumor virologists tried to show that tumor viruses were not different from ordinary viruses. Duran-Reynalls was one of the major figures in the attempt to establish this connection [4].

A return toward differentiation came in the late 50s and early 60s when RSV was shown to have genes able to alter specifically the morphology of infected cells. It was then shown that these genes could alter the differentiation of iris epithelial cells—turning off pigment production and starting fibroblast functions like hyaluronic-acid synthesis—and that they could alter the differentiation of fibroblasts, increasing

the activity of hyaluronic acid synthetase [5,6]. Implicit in these results was the concept that viral carcinogenesis was the result of introduction of a gene not involved in virus replication and specifying a pleiotropic effector molecule [7]. (However, these terms were not used then.)

At about the same time, Marcel Baluda presented results indicating to me that transformation by avian myeloblastosis virus (AMV) depended upon infection of target cells at a particular stage of differentiation, although AMV could replicate in other cells without transformation [8].

These studies have their present-day parallel in studies on the interaction of viral oncogene products with cell proteins and cell behavior.

THE PROTOVIRUS HYPOTHESIS

However, the contact of tumor viruses with McClintock's work came from other directions. In 1969 and 1970 I was writing a review on malignant transformation of cells by viruses for Perspectives in Biology and Medicine [9]. I discussed the evidence for the DNA provirus and then attempted to extend "this hypothesis in a speculative fashion to encompass the occurrence in the viral genome of information for a viral product controlling cell multiplication and to explain the spontaneous occurrence of such powerful oncogenic agents." I speculated that there might be in normal cells precursors of retroviruses—which I called protoviruses—that could give rise through transcription and reverse transcription to a DNA copy which was incorporated into the genome. Parenthetically, I stated "this system may be similar to [McClintock's] modifying elements in maize."

Remarkably, DNA cloning and sequencing have now established a striking structural similarity between retrovirus proviruses and cellular movable genetic elements, including presumably those in maize. For example, copia, a movable genetic element of Drosophilia, and spleen necrosis virus, an avian retrovirus related to primate retroviruses, have numerous structural and nucleotide sequence homologies (Fig. 1).

PRESENT KNOWLEDGE OF RETROVIRUSES

In addition to this resemblance to transposons, we know that retroviruses allow a great variety of different sequences between their ends. Retroviruses can contain coding genes for virus proteins (nondefective retroviruses), for transforming proteins (highly oncogenic retroviruses), or for other proteins (in vitro constructed recombinant retrovirus vectors) [11,12] (Fig. 2). We also know that retroviruses can infect germ line cells and become a heritable part of organisms (endogenous retroviruses).

Relative to differentiation, we know that cellular coding sequences under control of retrovirus control sequences can cause transformation in a pleiotropic fashion and that the effectiveness of the transformation usually depends upon the differentiated state of the appropriate target cell. These two processes are probably the major connection of RNA tumor viruses and differentiation.

In the remainder of this paper, I shall discuss the normal cell not infected with viruses and ask if there are protoviruses in these cells and, if so, what is their effect on cells.

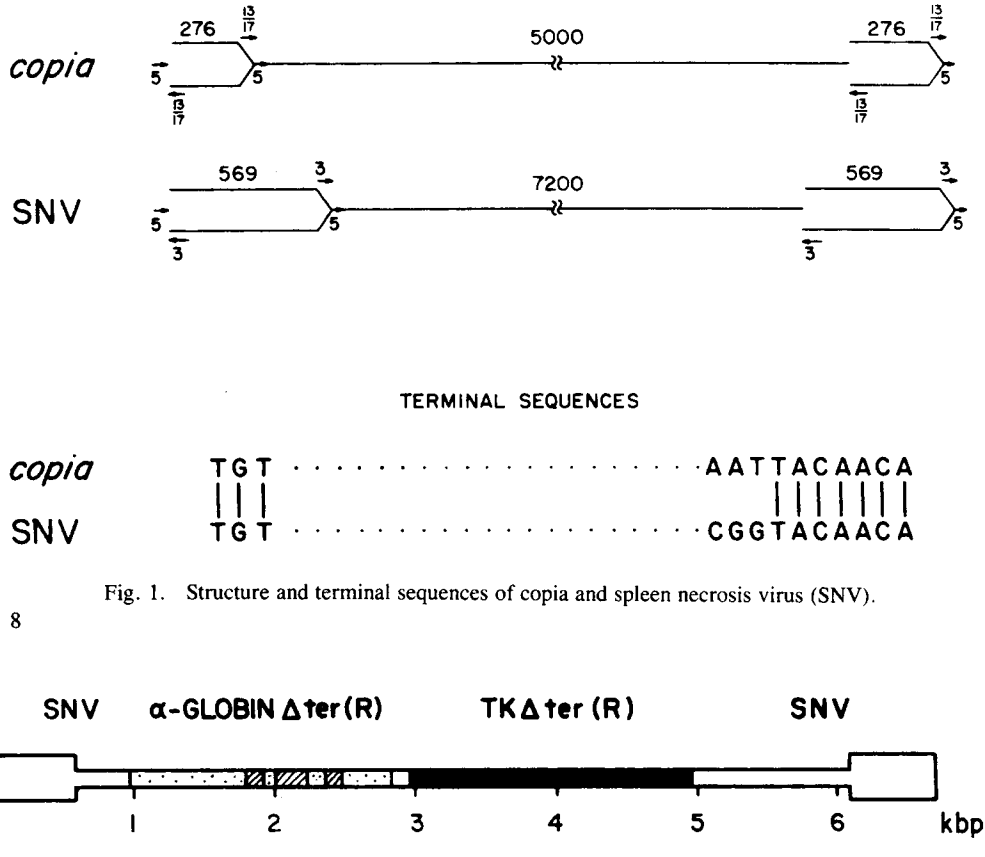

Fig. 1. Structure and terminal sequences of copia and spleen necrosis virus (SNV).

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Fig. 2. Map of TK- α-globin-SNV. A provirus of SNV containing 1 kbp of SNV; mouse α-globin △ ter(R); 300 bp of SNV; herpes simplex virus thymidine kinase gene △ ter(R); and 1 kbp of SNV plus a second LTR is shown (from [66]).

CANDIDATE PROTOVIRUSES IN VERTEBRATE DNA

There are several families of sequences in vertebrate DNA that could be protoviruses, ie, genetic elements that transpose by integration of a DNA copy of an RNA transcript of the element.

These families do not include most so-called endogenous retroviruses. Numerous recent work using Southern blot analysis clearly show that most endogenous proviruses are the result of fairly recent infection of the germ line [13]. Although there are still questions about these proviruses—why most of them are xenotropic, how they were amplified—they apparently have no role in cell or organismal development, except for those endogenous viruses responsible for late neoplasia, eg, AKR mouse leukemia virus and mouse mammary tumor virus. Both of these endogenous viruses, however, were selected for their ability to promote tumor formation in the creation of inbred mice strains.

The most likely protoviruses are the 30S viruslike sequences of mice and rats and the intracisternal A-particle sequences. In addition, there has been speculation

that some Alu family sequences and small nuclear RNA genes and other genes may have transposed through an RNA intermediate.

30S viruslike sequences have been found as RNA in virions of murine leukemia virus grown in certain mouse and rat cells [14]. 30S sequences can "infect" uninfected cells [15]. They also form part of the genome of Harvey and Kirsten sarcoma viruses. Thus, these sequences can be encapsidated in retrovirus proteins and reverse transcribed, and even integrated. In accordance with these observations, their DNA has a long terminal repeat (LTR) with sequences from the 3′end of the 30S RNA at both ends of the DNA that is probably similar to the LTR of infectious retroviruses [14]. Thus the terminal sequences, LTR, primers, and encapsidation sequences are retroviruslike. There are approximately 1,000 copies of these sequences in a normal cell, which is over 0.1% of the cell genome.

30S viruslike sequences are not related in nucleotide sequence to any exogenous retrovirus and they are reiterated to a greater extent than endogenous retroviruses. The high copy number coupled with dispersion through the genome indicate that either they are a cellular movable genetic element or integrated into a cellular movable genetic element.

It is not known how the 30S viruslike sequences were amplified. However, the existence in these sequences of a functional LTR and other terminal sequences involved in infectious virus formation indicates that reverse transcription (and encapsidation) may have been important in the amplification. Thus, 30S viruslike sequences may well be protoviruses.

A very similar argument can be made about intracisternal A-particle sequences. Intracisternal A-particles are noninfectious particles resembling immature B-type retroviruses, but found within the endoplasmic reticulum in several mouse tumor lines and in normal preimplantation mouse embryos [16]. There are over 1,000 genes homologous to intracisternal A-particle RNA and some of these genes contain LTRs functional in transcription. Although there is some sequence homology to a retrovirus—M432 from Mus cervicolor—it appears that the intracisternal A-particle sequences are an independent element and that the homology with M432 is the result of recombination of a preexisting retrovirus and the intracisternal A-particle sequences [17].

The same considerations raised above for 30S viruslike sequences are applicable to intracisternal A-particle sequences. However, although intracisternal A-particles contain a reverse transcriptase, they have not yet been shown to be able to form a new provirus. (But see [67].)

Several groups of authors have interpreted the results of sequencing genes for Alu family members, small nuclear RNAs, and some pseudogenes as indicating an RNA intermediate, reverse transcription, and integration as a mechanism for transposition and amplification [18,19]. The basis for this speculation is the presence of a direct repeat of 5 to 19 base pairs around the sequence, a 3′poly(A) stretch in the sequence, and in some cases, loss of intervening sequences compared to a homologous sequence in the same genome. Clearly these sequences are not as close to retroviruses as are 30S viruslike RNA sequences and intracisternal A-particles. Furthermore, transposition through an RNA intermediate, if it exists, might only have been a result of abnormal transcription of these genes. The origin of the needed reverse transcriptase and integrase are unclear and could not easily be coded by the Alu or small nuclear RNA sequences since they are very small (see below).

In addition, there are many other dispersed moderately reiterated sequences in the vertebrate genome whose mode of amplification is unknown. I assume that many of these reiterated sequences represent movable genetic elements. Furthermore, even "orthodox" movable genetic elements of eukaryotes may use reverse transcription. For example, unintegrated DNA of copia has been reported [20] and the two LTRs (δ) of Tyl are concordant in any one element but different from element to element (Fig. 3) [21,22], indicating some mechanism of information transfer between them, either the "jumping" of DNA in LTR formation during reverse transcription or gene conversion. For retroviruses, the existence of this information transfer has been directly shown by use of an artificially constructed recombinant virus with different LTRs [23]. Progeny viruses had both LTRs the same.

Finally, it must be added that vertebrate cells have very active constitutive or inducible systems for ligating DNA and perhaps also for cutting it. This system is operative in integration of papova- and adenovirus DNAs and in formation of concatamers and recombinants after DNA transfection [24,25]. It may also be involved in the amplification of some gene families. It does not involve sequence homology or result in surrounding direct repeats.

ROLES OF MOVABLE GENETIC ELEMENTS

The Adaptationist Hypothesis

Given the existence of movable elements and mechanisms for moving them, we next ask if movable genetic elements have a role in normal cell differentiation. In answering this question, however, we must not fall into what Gould and Lewontin [26] have called "the spandrels of San Marco and the Panglossian paradigm," that is, assuming that because any trait or character exists, it has adaptational (and, thus, selective) value for the organism. The discussion of "selfish DNA" revolves around this question.

Gould and Lewontin discuss other explanations for the presence of particular traits in an organism (Here the trait would be the presence in an organism of sequences of movable genetic elements): 1) no adaptation and no selection (ie, the movable genetic elements have no effect on the organism; they are neutral sequences); 2) no adaptation and no selection for these sequences, but their presence is a correlated

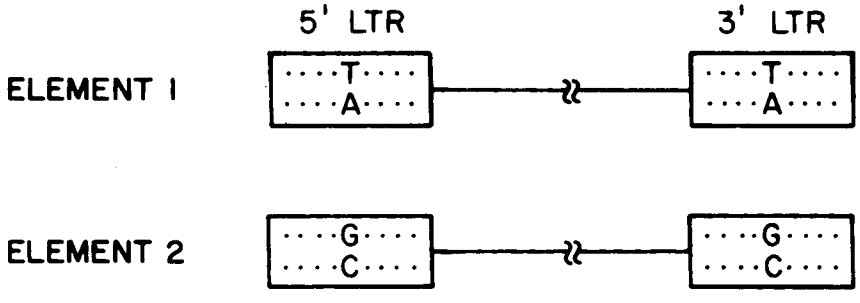

Fig. 3. Concordance of sequence of two LTRs of any one element. Two elements with LTRs are shown. Only where the sequences differ are they shown.

consequence of selection directed elsewhere; eg, the requirement for repair systems, recombination, etc; 3) selection for element without adaptation of organism; 4) adaptation and selection, but no selective basis for differences among adaptations, different numbers and types of elements; and 5) adaptation and selection, but the adaptation is a secondary utilization of sequences present for reasons of architecture, development or history, thus, the evolution of retroviruses from movable genetic elements.

These other possibilities must be kept in mind when we consider the presence of these elements in the genomes.

Rearrangements in DNA Primary Sequences and Differentiation

There are now numerous examples in the biological world of changes in DNA primary sequences affecting differentiation in the sense of causing changes in properties of cells. These include phase variation in Salmonella, mating type interconversion in yeast, antigenic variation in trypanasomes, macronuclear reorganization in ciliates, chromosome diminution in invertebrates, formation of immunoglobulins, and insertion of the retrovirus LTR [27–39]. It is not obvious how relevant these examples of DNA sequence rearrangements are to most cellular differentiation in vertebrates.

Most known DNA primary sequence rearrangements result in major alterations of the whole genome, eg, chromosome diminution in invertebrates, or alternate states that are usually reversible, eg, yeast-mating type or trypanasome-antigen type. Thus, most DNA primary sequence rearrangements may only be relevant to certain end-stages of cell differentiation or other special cases in vertebrates where reversible alternate states are useful.

In contrast, the formation of immunoglobulins involves progressive and irreversible changes in the DNA primary structure. The DNA changes occur in a controlled fashion presumably as a result of other events in differentiation. However, very special DNA structures and large DNA rearrangements are involved and the outcome of the rearrangements is often nonfunctional. Furthermore, the rearrangements are partly driven by antigens and the outcome apparently results from cellular selection by antigen. Formation of immunoglobulins may be a model for some stochastic forms of cellular differentiation triggered by specific environmental stimuli, eg, the rest of hematopoiesis, but more likely it is only an incredibly complex and sophisticated special type of cell differentiation.

Retrovirus insertion is another form of "differentiation" involving DNA primary sequence rearrangements. Apparently, integration is a completely random process. In the chicken, a specific "differentiation"—B-cell lymphoma—occurs months after infection by certain retroviruses apparently as a result of integration near a particular cellular gene [38,39]. However, this differentiation appears to be selected by its neoplastic behavior and to be quite rare in terms of the total number of infected cells. Furthermore, no precise regulation of time or end point is present.

Important as these examples of DNA primary sequence rearrangements and differentiation are, I now find it hard to generalize them into a mechanism of vertebrate cell differentiation. Of course, we still might find that DNA primary sequence rearrangements accompany and stabilize most cell differentiation. If such rearrangements were to occur, we would then need to know what controls the occurrence of the sequence rearrangements. It is not hard to imagine an answer to this question. Then we would need to know what determines the specificity of the

DNA sequence rearrangements, a harder question to answer. So far, the major ways of getting specificity of DNA sequence rearrangements are through specific integration or transposition and selection. Together these processes could be sufficient to determine the specificity of rearrangements in cell differentiation. However, it is easier to imagine the existence of specific integration when the sequence complexity is that of a bacterium rather than a vertebrate cell. Furthermore, succeeding DNA primary sequence rearrangements would apparently require expression of different integrases or transposases, each of which would also have to be controlled in some way. (Differentiation involving changes in amounts of protein factors also involves this unending problem of dolls within dolls.)

Changes Not Involving Rearrangements in DNA Primary Sequence

There are changes in portions of chromosomes and DNA that do not involve rearrangements of DNA primary sequence. The most discussed mechanisms at present are gene amplification, methylation, and changes resulting in DNase-sensitivity [40,41]. Perhaps attachment to the nuclear matrix will also be important [42]. In addition, at another level of organization, inactivation of the X-chromosome (which may also involve methylation) and heterochromatization of some chromosomal regions also occur [43].

These processes apparently provide stable ways of changing DNA activity and, consequently, cellular differentiation, without DNA primary sequence rearrangements. Of course, for these mechanisms there still is the fundamental problem of control of time of occurrence and specificity of changes in the DNA and chromosomes.

Studies of endogenous proviruses appear to indicate that there is regional and temporal, but not DNA primary sequence control of the specificity of methylation and induction which does not initially involve DNA primary sequence rearrangements [44–46]. (We earlier called this phenomenon cis-acting control elements [47].) What I mean by this is that once an inactive endogenous provirus is activated by an unknown mechanism, virus is produced. This virus results in reinfection and integration at a new location in the genome from the inactive provirus. Because the new provirus is active and produces virus, a new stable differentiation—virus production—is therefore produced.

If this process is a model for differentiation, DNA rearrangements would stabilize a new state of differentiation after the rearrangement is triggered by other events. (In the case of activation of a provirus and reinfection, DNA rearrangement leads to a new phenotype. However, I discuss this example in terms of what it tells us about the control of methylation, etc, when there is no DNA primary sequence rearrangements.)

An argument against a scenario involving DNA rearrangements in normal cellular differentiation is the apparent reversibility of some differentiation. In particular, some of the nuclear transplants from apparently differentiated cells to enucleated frog eggs and the implantation of some teratoma cells into blastocysts with normal later frog or mouse development is interpreted as indication that cell differentiation and neoplasia are reversible [48,49]. However, DNA rearrangements can be reversible, eg, the precise excision of transposons [50]. Alternatively, early embryos may have a way—perhaps the hypermethylation of all sequences—to negate (inactivate) any differentiation caused by DNA rearrangements. Only those sequences pro-

grammed to be demethylated in normal differentiation would be active later, thus, the DNA primary sequence rearrangements might remain but be inactive.

TUMOR VIRUS PRODUCTS AND DIFFERENTIATION

The experiments with endogenous proviruses indicate one impact of tumor virology on studies of differentiation. The different locations of proviruses with different patterns of activation provide markers for regions of chromosomes with different patterns of activation. The provirus can be used as a tag to clone DNA sequences from regions with interesting specificity of activation.

Another area in which tumor viruses affect differentiation is with respect to the products of highly oncogenic retroviruses. A great deal of evidence shows that many integration sites of *onc*-containing proviruses in the genome are compatable with the full effectiveness of the viruses as transforming agents [11]. In these cases, the viruses either cause abnormal differentiation in the same pathway, eg, acute leukemia viruses, or a completely abnormal differentiation, eg, sarcoma viruses in nonfibroblastic cells. The abnormal differentiation usually involves continued cell multiplication.

Most interesting in this respect is MC29 virus, which in its original isolations resulted in a particular type of leukemia involving macrophage precursors. However, MC29 virus also causes liver and kidney carcinomas. Apparently, the product of the *myc* viral oncogene can cause an abnormal macrophage differentiation when in one appropriate target cell and carcinomas when in other target cells. (More puzzling is the postulated role of an activated *c-myc* gene in B-cell lymphomas [38,39].)

A lesson for differentiation is that the activity of a single gene can have major pleiotropic effects on differentiation, but the nature of the effects depends upon what other genes are active in the cell. Thus, to understand the genetic basis of differentiation, ie, if and what primary DNA sequence rearrangements have occurred, it might be necessary first to know the key products controlling differentiation and their genes.

Protooncogenes may code for such key products controlling differentiation. Then tumor viruses will not only give us a way to understand neoplasia, but also a way to understand differentiation by isolating the key genes for at least certain types of differentiation.

EVOLUTION

Although DNA rearrangements, movable genetic elements, and repetitive DNA may have little to do directly with most vertebrate cell differentiation, DNA rearrangements, especially gene duplication, are an important mechanism in evolution. It is here rather than in differentiation that movable genetic elements may have their most important effects on organisms.

These assertions would probably be accepted by most people. Most relevant is the question of whether protoviruslike elements have a role in evolution and if movable genetic elements are the elements that led to rapid evolution without gross DNA primary sequence changes. One could even extend this question to encompass whether or not viruses themselves, not just cellular movable genetic elements, are the motive force behind some rapid evolution and speciation. Certainly one way to increase drastically the rate of genetic change would be to make germ line cells sensitive to retrovirus infection. The infections would lead to gene activations and

inactivations, and perhaps rearrangements and deletions as well. Ultimately, there would be selection for resistance to such infection, but the genetic changes would already have occurred.

I have already mentioned the possible role of RNA intermediates in some amplification of Alu and small nuclear RNA gene families(s) [18,19]. A similar possibility has been raised in the formation of intron-less gene duplicates, for example, α-globin and tubulin pseudogenes [51–55]. The simplest way to imagine stable removal of intervening sequences is transcription, processing, reverse transcription, and integration. Unfortunately, the more we learn about retrovirus replication, the less likely it is that just any mRNA could be reverse transcribed and integrated [56]. Retrovirus DNA synthesis requires two separate primers at least. One involves a specific host tRNA annealed to 16–18 bases near the 5′end of viral RNA; the other, apparently a purine run at the 3′end of viral RNA. Two nucleases are involved—ribonuclease H and an endonuclease to release the + strand primer. Two jumps of viral DNA and polymerase also seem to occur; perhaps this is why the virus always has two copies of viral RNA. A provirus clone we sequenced shows jumps from two RNA molecules as well as a random formation of a primer with no apparent homology (Fig. 4) [57].

Integration appears to involve specific sequences at the end of viral DNA including TG....CA in an inverted repeat and formation of a small (4 to 6 base pair) direct repeat in cell DNA at the site of integration [10]. One or two specific nucleases and a ligase would be needed for integration.

Thus, it is not possible to ignore these requirements in considering cDNA genes. One possibility is that the mRNA was inserted into a retrovirus genome [58]. But this hypothesis imposes certain polarity requirements and not merely propinquity of the pseudogene and a retrovirus LTR. Alternatively, one can consider that for retroviruses reverse transcription and integration must provide a DNA copy that contains all of the sequences in the RNA and immediately surrounding sequences. Since gene duplication does not have this requirement, a simpler form of primer could be imagined. For example, loop back of a processed transcript could provide a primer for cDNA synthesis. However, + strand DNA synthesis also requires a primer. If the RNA template were removed by ribonuclease H except at the 5′end, an RNA would remain (Fig. 5). (Alternatively, the cDNA could form a hairpin loop at its end that acts as a primer.) Presumably some nuclease to make staggered cuts in cell DNA and ligase would complete the process.

This scenario requires several enzyme activities not usually found in vertebrate cells as well as inefficient primer formation. However, since we are talking about evolutionary time rather than developmental time, these processes could be very inefficient. In addition, the variation in size of direct repeats (Table I) could be a result of mutation. The large size of direct repeats compared to those of retroviruses and the lack of TG....CA ends indicates that a different enzyme activity was used than is now used by retroviruses. Since the transpositions happened a long time ago, that enzyme may no longer exist, although the enzyme used in retrovirus integration may be descended from it.

This hypothesis also leaves the problem of integration. However, it appears that cell DNA is very efficient in integration or ligation of unintegrated DNA. Originally, this may have been a mechanism to repair double-stranded DNA breaks, but analysis of DNA tumor virus integration and, even more, analysis of DNA after transfection,

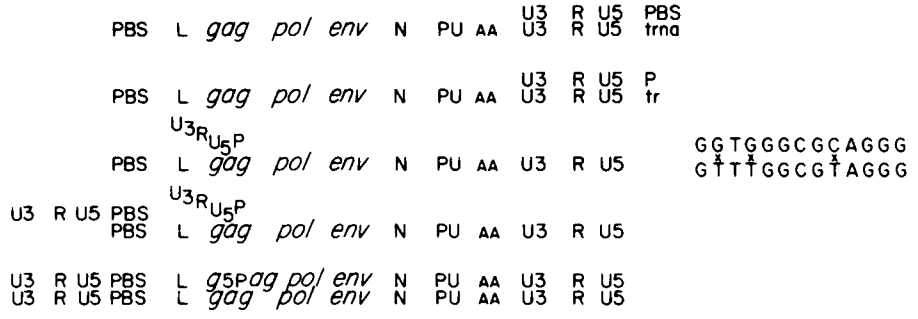

Fig. 4. Formation of a variant SNV provirus. Steps in synthesis of DNA of a variant SNV provirus with a non tandem duplication are shown [57]. Abbreviations are as in [10].

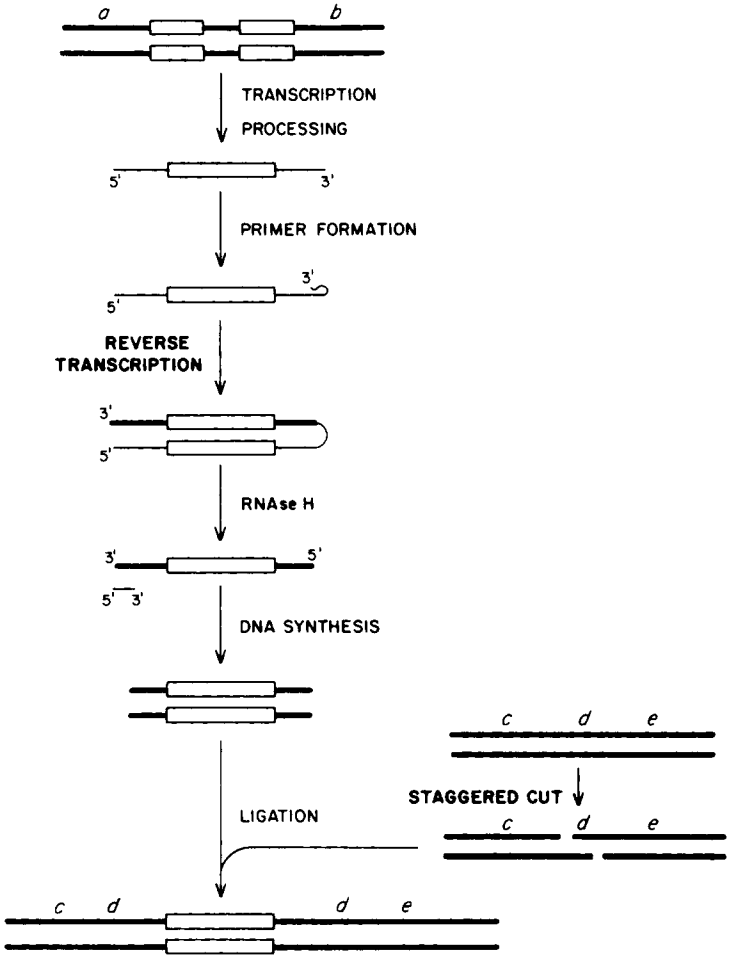

Fig. 5. Hypothesis of formation of cDNA genes.

TABLE I. Size of Cellular Direct Repeats

Retroviruses	
Murine leukemia virus	4, (5)
Spleen necrosis virus	5
Avian leukosis virus	6
Mouse mammary tumor virus	6
Movable genetic elements	
copia	5
Tyl	5
Candidate cDNA genes	
α-Globin pseudogene	?
Alu	5,7,8,10,14, 15,17,18,19
Immunoglobulin light chain pseudogene	9
Tubulin pseudogenes	11, 15
snRNA pseudogenes	16,18,19

See [10, 11, 18–22, 51–65].

indicate that active ligases and nucleases must exist in some cells [24,25]. Thus, as long as specificity of integration is not required, DNA copies might be relatively efficiently integrated. The frequency of such integration might be too low to be used as a mechanism for differentiation, but, if anything, that might be an advantage for thinking about this type of rearrangement as a mechanism for gene duplication in evolution.

The missing link in the connection between tumor viruses and cellular movable genetic elements and the genetics of differentiation is specificity of integration. In its absence, I am relatively pessimistic that DNA rearrangement is the primary mechanism of cell differentiation.

In conclusion, I think that McClintock's controlling elements, tumor viruses, and protoviruses are elements more active in evolution than in normal differentiation. Of course, cancer involves abnormal differentiation, and here I think it is very likely that these elements have a major role. The major differences are that there are many cells at risk of cancer, and no precise regulation of time or end point is required. In addition, the products of viral oncogenes and protooncogenes may provide major models to understand the physiology of differentiation.

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